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A novel polymorphism of the CYP2J2 gene is associated with coronary artery disease in Uygur population in China

Qing Zhu [1], Zhenyan Fu [1], Yitong Ma *, Hong Yang, Ding Huang, Xiang Xie, Fen Liu, Yingying Zheng, Erdenbat Cha

Department of Cardiovascular Medicine, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

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ABSTRACT

Background: Cytochrome P450 (CYP) 2J2 is expressed in the vascular endothelium and metabolizes arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs). The EETs are potent endogenous vasodilators and inhibitors of vascular inflammation. The aim of the present study was to assess the association between the human CYP2J2 gene polymorphism and coronary artery disease (CAD) in a Han and Uygur population of China.

Methods: We use two independent case–control studies: a Han population (206 CAD patients and 262 control subjects) and an Uygur population (336 CAD patients and 448 control subjects). All CAD patients and controls were genotyped for the same three single nucleotide polymorphisms (SNPs) (rs890293, rs11572223 and rs2280275) of CYP2J2 gene by a real-time PCR instrument.

Results: In the Uygur population, for total, the distribution of SNP3 (rs2280275) genotypes showed a significant difference between CAD and control participants ($P = 0.048$). For total and men, the distribution of SNP3 (rs2280275) alleles and the dominant model (CC vs CT + TT) showed a significant difference between CAD and control participants (for allele: $P = 0.014$ and $P = 0.035$, respectively; for dominant model: $P = 0.014$ and $P = 0.034$, respectively). The significant difference in dominant model was retained after adjustment for covariates (OR: 0.279, 95% confidence interval [CI]: 0.176–0.440, $P = 0.001$; OR: 0.240, 95% CI: 0.128–0.457, $P = 0.001$, respectively).

Conclusions: The CC genotype of rs2280275 in CYP2J2 gene could be a protective genetic marker of CAD and T allele may be a risk genetic marker of CAD in men of Uygur population in China.

1. Introduction

Coronary artery disease (CAD) is a complex multifactorial and polygenic disorder thought to result from an interaction between an individual's genetic makeup and different environments [1,2]. Various gene variants have been shown to be associated with CAD [3,4]. The cytochromep450 (CYP) enzymes are a super-family of cysteinato-heme enzymes [5,6]. They are responsible for not only the metabolism of xenobiotics but also a host of endogenous substance whose metabolites have critical roles in the maintenance of cardiovascular health [7,8]. Mounting evidences have demonstrated that CYP enzymes are involved in the pathogenesis of CAD. Polymorphisms of CYP genes, for example, CYP1A1, CYP1A2, and CYP1B1 (metabolize tobacco-smoke polycyclic aromatic hydrocarbons and 17-beta-estradiol) [9–11], CYP2C8, CYP2C9, and CYP2J2 (synthesis of EETs) [12–14], CYP2C19 (involved in clopidogrel metabolism) [15], CYP3A4 (main metabolic enzyme of fentanyl) [16], CYP4A11 and CYP4F2 (synthesis of 20-hydroxyeicosatetraenoicacid) [17,18], CYP8A (prostacyclin synthesis) [19], and CYP11B2 (aldosterone synthesis) [20] have been demonstrated to have a relationship with CAD. In the human heart, EETs are mainly produced by CYP2J2 [21], which play an important role in the regulation of cardiovascular inflammation [22], and possess potent vasodilatory, antiapoptotic properties in the cardiovascular system [14,23,24]. Common polymorphisms within CYP2J2 can result in the variation of EETs, which may determine susceptibility to the development of cardiovascular disease.

Recently, some reports on the cardiovascular risk associated with CYP2J2 polymorphisms have provided inconsistent results. The studies by Spiecker M, Liu PY and Lee showed that a polymorphism of the CYP2J2 gene (rs890293) was associated with CAD and MI [14,25,26]. There were several studies suggesting no significant association between the polymorphism of CYP2J2 (rs890293) and CAD or MI [27–29]. Based on the above background, we aimed to assess the association between the polymorphism of CYP2J2 and CAD in a Han and Uygur population of China.

* Corresponding author at: Department of Cardiovascular Medicine, First Affiliated Hospital of Xinjiang Medical University, Li Yu Shan South Road 137, Urumqi 830054, China. Fax: +86 991 4366191.
E-mail address: myt-xj@163.com (Y. Ma).
[1] These authors contributed equally to this work.

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2. Methods

2.1. Ethical approval of the study protocol

Written informed consent was obtained from all participants. All participants explicitly provided permission for DNA analyses as well as collection of relevant clinical data. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China). It was conducted according to the standards of the Declaration of Helsinki.

2.2. Subjects

The subjects were from a Han population and an Uygur population who lived in the Xinjiang Uygur Autonomous Region of China. All patients and controls had a differential diagnosis for chest pain encountered in the Cardiac Catheterization Laboratory of First Affiliated Hospital of Xinjiang Medical University from 2006 to 2012. All of the procedures of coronary angiography were undertaken by highly skilled physicians using the Judkins approach. The findings of coronary angiography were interpreted by a least two experienced imaging specialists and the final diagnosis of CAD was made according to the angiography report.

We recruited randomly 206 Han patients and 336 Uygur patients with CAD and 262 and 448 ethnically and geographically matched control group. All CAD groups defined as the presence of at least one significant coronary artery stenoses of more than 50% luminal diameter on coronary angiography. All control subjects also underwent a coronary angiogram and have no coronary artery stenoses and did not show clinical or electrocardiogram evidence of myocardial infarction (MI) or CAD. Control subjects were not healthy individuals, some of them have hypertension, some of them have DM, and some of them have hyperlipidemia, which means control group expose to the same risk factors of CAD while the results of coronary angiogram are normal. Data and information about traditional coronary risk factors, including hypertension, diabetes mellitus (DM), and smoking, were collected from all study participants. The diagnosis of hypertension was established if patients were on antihypertensive medication or if the mean of 3 measurements of systolic blood pressure (SBP) >140 mm Hg or diastolic blood pressure (DBP) >90 mm Hg, respectively. Diabetes mellitus was diagnosed according to the criteria of the American Diabetes Association [30]. In addition, individuals with fasting plasma glucose >7.0 mmol/L or with a history of diabetes or treatment with insulin were considered diabetic. Smoking was classified as smokers (including current and ex-smokers) or non-smokers. All patients with impaired renal function, malignancy, connective tissue disease, valvular disease or chronic inflammatory disease were excluded.

2.3. Blood collection and DNA extraction

Fasting blood samples drawn by venipuncture in the catheter-room were taken from all participants before cardiac catheterization. The blood samples were drawn into a 5 mL ethylene diamine tetraacetic acid (EDTA) tube and centrifuged at 4000 ×*g* for 5 min to separate the plasma content. Genomic DNA was extracted from the peripheral leukocytes using standard phenol–chloroform method. The DNA samples were stored at −80 °C until use. While used, the DNA was diluted to 50 ng/μL concentration.

2.4. Genotyping

There are 701 SNPs for the human CYP2J2 gene listed in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP). Using the Haploview 4.2 software and the HapMap phrase II database, we obtained three tag SNPs (rs890293, rs11572223 and rs2280275) by using minor allele frequency (MAF) ≤0.1 and linkage disequilibrium patterns with $r^2 \geq 0.5$ as a cut off. The position of the SNP1, SNP2 and SNP3 (rs890293, rs11572223 and rs2280275) was by order of increasing distance from the CYP2J2 gene 5′ end (Fig. 1). SNP1 (rs890293) was observed in the proximal promoter region of the gene. The polymorphisms caused a loss of transcription factor binding site Sp1.

Genotyping was undertaken using the TaqMan® SNP Genotyping Assay (Applied Biosystems). The primers and probes used in the TaqMan® SNP Genotyping Assays (ABI) were chosen based on information at the ABI website (http://myscience.appliedbiosystems.com). Thermal cycling was done using the Applied Biosystems7900HT Standard Real-Time PCR System. Plates were read on Sequence Detection Systems (SDS) automation controller software v2.3 (ABI). PCR amplification was performed using 2.5 μL of TaqMan Universal Master Mix, 0.15 μL probes and 1.85 ddH_2O in a 6-μL final reaction volume containing 1 μL DNA. Thermal cycling conditions were as follows: 95 °C for 5 min; 40 cycles of 95 °C for 15 s; and 60 °C for 1 min. All 96 well plates were read on Sequence Detection Systems (SDS) automation controller software v2.3 (ABI).

2.5. Biochemical analysis

Serum concentrations of total cholesterol (TC), triglyceride (TG), glucose (Glu), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were measured using standard methods in the Central Laboratory of First Affiliated Hospital of Xinjiang Medical University.

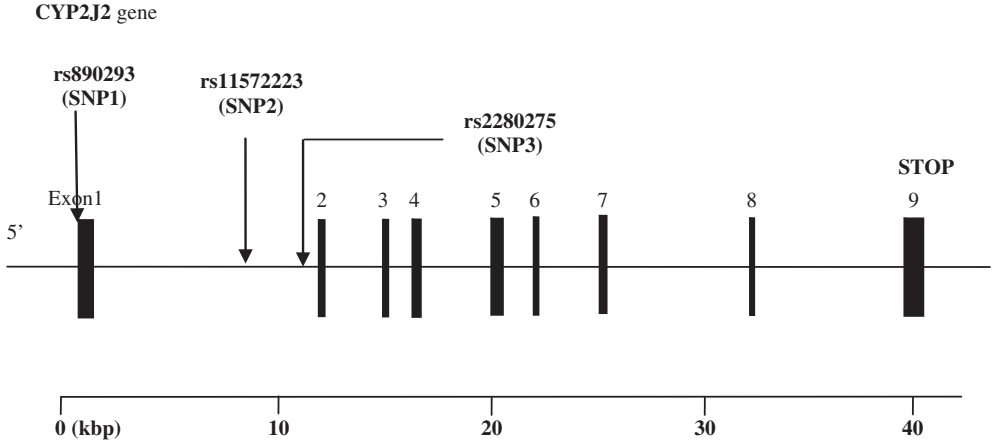

Fig. 1. Structure of the human CYP2J2 gene. This gene consists of 9 exons separated by 8 introns. Boxes indicate exons, and lines indicate introns and intergenic regions. Filled boxes indicate coding regions. Arrows mark the locations of polymorphisms.

Table 1
Characteristics of study participants.

	Han									Uygur								
	Total			Men			Women			Total			Men			Women		
	CAD patients	Control subjects	P value	CAD patients	Control subjects	P value	CAD patients	Control subjects	P value	CAD patients	Control subjects	P value	CAD patients	Control subjects	P value	CAD patients	Control subjects	P value
Number (n)	206	261		156	141		50	120		336	448		272	186		64	262	
Age (years)	58.69 ± 7.50	57.76 ± 8.01	0.401	59.97 ± 8.119	58.60 ± 8.51	0.231	58.13 ± 6.28	59.85 ± 6.89	0.061	55.97 ± 5.75	54.93 ± 5.59	0.076	55.57 ± 8.32	53.96 ± 9.05	0.090	53.20 ± 3.88	51.30 ± 4.82	0.066
BMI (kg/m^2)	27.73 ± 3.84	28.04 ± 2.85	0.349	26.54 ± 1.89	26.23 ± 1.82	0.175	30.76 ± 1.84	30.06 ± 2.10	0.054	31.34 ± 7.19	27.11 ± 4.36	0.001*	30.45 ± 6.83	27.48 ± 3.97	0.001*	31.97 ± 7.39	25.73 ± 5.40	0.001*
Pulse (beats/min)	73.16 ± 10.12	73.72 ± 10.02	0.554	73.19 ± 10.43	74.77 ± 11.16	0.213	73.06 ± 9.18	72.51 ± 8.40	0.706	75.59 ± 10.91	77.56 ± 7.92	0.390	74.49 ± 11.21	75.76 ± 8.46	0.733	77.91 ± 9.75	81.38 ± 5.18	0.339
Glu (mmol/L)	6.08 ± 2.41	5.44 ± 1.56	0.001*	5.79 ± 1.71	5.37 ± 1.33	0.024*	6.37 ± 3.07	5.30 ± 1.44	0.001*	6.50 ± 2.79	5.14 ± 2.39	0.001*	6.31 ± 2.63	5.10 ± 2.54	0.001*	7.21 ± 3.24	5.17 ± 2.30	0.001*
TG (mmol/L)	1.97 ± 1.32	1.98 ± 1.84	0.951	1.81 ± 0.92	1.67 ± 0.79	0.190	2.17 ± 1.76	1.79 ± 1.09	0.115	1.83 ± 1.25	1.76 ± 1.35	0.435	1.77 ± 0.99	1.83 ± 1.50	0.638	2.10 ± 2.07	1.70 ± 1.24	0.061
TC (mmol/L)	4.18 ± 1.06	4.32 ± 0.95	0.169	4.09 ± 1.03	4.22 ± 0.93	0.313	4.45 ± 1.14	4.44 ± 0.90	0.960	4.14 ± 1.07	4.05 ± 0.99	0.240	4.08 ± 1.05	4.03 ± 0.96	0.564	4.40 ± 1.14	4.07 ± 0.96	0.027*
HDL (mmol/L)	1.08 ± 0.33	1.15 ± 0.33	0.027	1.05 ± 0.28	1.18 ± 0.45	0.535	1.18 ± 0.45	1.23 ± 0.32	0.416	0.99 ± 0.23	1.02 ± 0.22	0.143	0.98 ± 0.30	0.99 ± 0.24	0.738	1.054 ± 0.30	1.05 ± 0.20	0.802
LCL (mmol/L)	2.47 ± 0.88	2.16 ± 0.90	0.001*	2.44 ± 0.84	2.11 ± 0.81	0.001*	2.50 ± 0.92	2.18 ± 0.97	0.001*	2.76 ± 0.85	2.49 ± 0.96	0.001*	2.80 ± 0.83	2.46 ± 0.98	0.001*	2.74 ± 0.86	2.62 ± 0.88	0.355
EH (%)	58.25	46.56	0.012*	60.50	42.29	0.002*	56.00	50.83	0.022*	41.67	17.41	0.001*	33.34	19.30	0.001*	50.00	15.52	0.001*
DM (%)	25.73	12.21	0.001*	20.46	14.42	0.001*	32.00	10.00	0.001*	25.60	11.83	0.001*	20.52	6.72	0.001*	29.68	16.94	0.001*
Smoke (%)	59.22	33.97	0.001*	86.44	55.94	0.001*	12.00	2.5	0.001*	32.14	16.96	0.001*	62.72	33.89	0.001*	1.56	0	–

BMI, body mass index; BUN, blood urea nitrogen; Cr, creatinine; Glu, glucose; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; EH, essential hypertension; DM, diabetes mellitus.
Continuous variable were expressed as mean ± standard deviation. P value of continuous variables was calculated by independent T-test.
The P value of categorical variable was calculated by Fisher's exact test. *$P < 0.05$.

2.6. Statistical analysis

All continuous variables (e.g. age, BMI, pulse, cholesterol levels) are presented as means ± standard deviation (S.D.). The difference between the CAD and control groups was analyzed using an independent-sample T-test. The differences in the frequencies of smoking, hypertension, diabetes mellitus, and CYP2J2 genotypes were analyzed using χ^2 test or Fisher's exact test while appropriate. Hardy–Weinberg equilibrium was assessed by χ^2 analysis. Logistic regression analyses with effect ratios (odds ratio [OR] and 95% CI) were used to assess the contribution of the major risk factors. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Institute, Chicago, USA). P-values of less than 0.05 were considered to statistically significant.

3. Results

Table 1 shows the clinical characteristics of the study participants. For Han and Uygur populations, there was no significant difference in age between CAD patients and control subjects. It means the study was an age-matched case–control study. In Han population, for total, men, and women, the plasma concentration of Glu, and LDL-C and the prevalence of essential hypertension (EH), diabetes mellitus (DM), and smoking were significantly higher for patients with CAD than for control participants. In Uygur population, for total and men, BMI, the plasma concentration of Glu, and LDL-C and the prevalence of EH, DM, and smoking were significantly higher for patients with CAD than for control participants. For women, BMI, the plasma concentration of Glu, and TC and the prevalence of EH, DM, and smoking were significantly higher for patients with CAD than for control participants.

Tables 2a and 2b show the distribution of genotypes and alleles of SNP1, SNP2 and SNP3 for the CYP2J2 gene (a: Han population; b:Uygur population). In the Uygur population, for total, the distribution of SNP3 (rs2280275) genotypes and additive model showed a significant difference between CAD and control participants (P = 0.048 and P = 0.027 respectively). For total and men, the distribution of SNP3 (rs2280275) alleles and the dominant model (CC vs CT + TT) showed a significant difference between CAD and control participants (for allele: P = 0.014 and P = 0.035, respectively; for dominant model: P = 0.014 and P = 0.034, respectively). T allele of rs2280275 was significantly higher in CAD patients than in control participants (total: 18.90% vs 12.29%; men: 18.57% vs 13.35%). The dominant model (CC vs CT + TT) of rs2280275 was significantly lower in CAD patients than in control participants (total: 66.96% vs 75.00%; men: 66.91% vs 75.27%). For the Han population, the distribution of the three SNP genotypes and alleles showed no significant difference between the CAD patients and control subjects.

Table 3 shows that multiple logistic regression analyses were done with Glu, LDL-C, EH, DM, and smoking because these variables were the major confounding factors for CAD. For total and men, the significant difference of rs2280275 was retained after adjustment for Glu, LDL-C, EH, DM, and smoking in Uygur population (for total participants, OR: 0.279, 95% confidence interval [CI]: 0.176–0.440, P = 0.001; and for men, OR: 0.240, 95% CI: 0.128–0.457, P = 0.001).

4. Discussion

Several CYP enzyme families have been identified in the heart, endothelium and smooth muscle of blood vessels. Increasing evidence indicates the role of endogenous CYP metabolites such as EETs, 20-hydroxyeicosatetraenoicacid, prostacyclin (PGI2), aldosterone and sex hormones in the maintenance of cardiovascular health. A link between the expression and activity of CYP and cardiovascular

Table 2a
Genotype and allele distributions in patients with CAD and control subjects (Han population).

				Total			Men			Women		
				CAD n (%)	Control n (%)	P value	CAD n (%)	Control n (%)	P value	CAD n (%)	Control n (%)	P value
rs890293 (SNP1)	Genotype	Dominant model	G/G	521 (90.92)	411 (90.33)	–	353 (89.59)	231 (90.23)	0.792	168 (93.85)	180 (90.45)	0.149
			T/T	1 (0.17)	0 (0.00)		0 (0.00)	0 (0.00)		1 (0.56)	0 (0.00)	
			G/T	51 (8.90)	44 (9.67)		41 (10.41)	25 (9.77)		10 (5.59)	19 (9.55)	
			GG	521 (90.92)	411 (90.33)	0.745	353 (89.59)	231 (90.23)	0.792	168 (93.85)	180 (90.45)	0.153
			GT + TT	52 (9.08)	44 (9.67)		41 (10.41)	25 (9.77)		11 (6.15)	19 (9.55)	
		Recessive model	TT	1 (0.17)	0 (0.00)	0.373	0 (0.00)	0 (0.00)	–	1 (0.56)	0 (0.00)	0.474
			GT + GG	572 (99.83)	455 (100.00)		353 (89.59)	231 (90.23)		178 (99.44)	199 (100.00)	
		Additive model	GT	51 (8.90)	44 (9.67)	0.672	41 (9.43)	25 (9.77)	0.823	10 (5.59)	19 (9.55)	0.149
			GG + TT	522 (91.10)	411 (90.33)		394 (90.57)	231 (90.23)		169 (94.41)	180 (90.45)	
	Allele		G	1093 (95.38)	866 (95.16)	0.823	747 (94.80)	489 (95.14)	0.785	346 (96.65)	379 (95.23)	0.325
			T	53 (4.62)	44 (4.84)		41 (5.20)	25 (4.86)		12 (3.35)	19 (4.77)	
rs11572223 (SNP2)	Genotype	Dominant model	C/C	158 (59.62)	232 (53.83)	0.326	124 (59.33)	130 (54.39)	0.574	34 (60.71)	102 (53.13)	0.531
			T/T	18 (6.79)	33 (7.66)		13 (6.22)	17 (7.11)		5 (8.93)	16 (8.33)	
			C/T	89 (33.58)	166 (38.52)		72 (34.45)	92 (38.49)		17 (30.36)	74 (38.54)	
			CC	158 (59.62)	232 (53.83)	0.135	124 (59.33)	130 (54.39)	0.293	34 (60.71)	102 (5.13)	0.315
			CT + TT	107 (40.38)	199 (46.17)		85 (40.67)	109 (45.61)		22 (39.29)	90 (46.88)	
		Recessive model	TT	18 (6.79)	33 (7.66)	0.671	13 (6.22)	17 (7.11)	0.609	5 (8.93)	16 (8.33)	1
			CT + CC	247 (93.21)	398 (92.34)		137 (65.55)	147 (61.51)		51 (91.07)	176 (91.67)	
		Additive model	CT	89 (33.58)	166 (38.52)	0.19	72 (34.45)	92 (38.49)	0.375	17 (30.36)	74 (38.54)	0.264
			CC + TT	176 (66.42)	265 (61.48)		137 (65.55)	147 (61.51)		39 (69.64)	118 (61.46)	
	Allele		C	405 (76.42)	630 (73.09)	0.167	320 (76.56)	352 (73.64)	0.315	85 (83.33)	278 (72.40)	0.462
			T	125 (23.58)	232 (26.91)		98 (23.44)	126 (26.36)		27 (26.47)	106 (27.60)	
rs2280275 (SNP3)	Genotype	Dominant model	C/C	167 (81.07)	204 (77.86)	0.567	125 (80.13)	110 (77.46)	0.709	42 (84.00)	94 (78.33)	0.795
			T/T	2 (0.97)	5 (1.91)		1 (0.64)	2 (1.41)		1 (2.00)	3 (2.50)	
			C/T	37 (17.96)	53 (20.23)		30 (19.23)	30 (21.13)		7 (14.00)	23 (19.17)	
			CC	167 (81.07)	204 (77.86)	0.396	125 (80.13)	110 (77.46)	0.574	42 (84.00)	94 (78.33)	0.4
			CT + TT	39 (18.93)	58 (22.14)		31 (19.87)	32 (22.54)		8 (16.00)	26 (21.67)	
		Recessive model	TT	2 (0.97)	5 (1.91)	0.395	1 (0.64)	2 (1.41)	0.607	1 (2.00)	3 (2.50)	1
			CT + CC	169 (82.04)	209 (79.77)		155 (99.36)	140 (98.59)		49 (98.00)	117 (97.50)	
		Additive model	CT	37 (17.96)	53 (20.23)	0.537	30 (19.23)	30 (21.13)	0.684	7 (14.00)	23 (19.17)	0.421
			CC + TT	169 (82.04)	209 (79.77)		126 (80.77)	112 (78.87)		43 (86.00)	97 (80.83)	
	Allele		C	371 (90.05)	461 (87.98)	0.317	280 (89.74)	250 (88.03)	0.525	91 (91.00)	211 (87.92)	0.456
			T	41 (9.95)	63 (12.02)		32 (10.26)	34 (11.97)		9 (9.00)	29 (12.08)	

CAD, coronary artery disease.
The P value of genotype was calculated by Fisher's exact test. *P < 0.05.

Table 2b
Genotype and allele distributions in patients with CAD and control subjects (Uygur population).

				Total			Men			Women		
				CAD n (%)	Control n (%)	P value	CAD n (%)	Control n (%)	P value	CAD n (%)	Control n (%)	P value
rs890293 (SNP1)	Genotype	Dominant model	G/G	253 (88.46)	125 (90.58)	0.669	213 (88.75)	82 (90.11)	0.773	40 (86.96)	43 (89.58)	0.692
			T/T	1 (0.35)	0 (0.00)		1 (0.42)	0 (0.00)		0 (0.00)	0 (0.00)	
			G/T	32 (11.19)	13 (9.42)		26 (10.83)	8 (8.79)		6 (13.04)	5 (10.42)	
			GG	253 (88.46)	125 (90.58)	0.511	213 (88.75)	82 (90.11)	0.535	40 (86.96)	43 (89.58)	0.692
			GT + TT	33 (11.54)	13 (9.42)		27 (11.25)	8 (8.79)		6 (13.04)	5 (10.42)	
		Recessive model	TT	1 (0.35)	0 (0.00)	0.487	1 (0.42)	0 (0.00)	1	0 (0.00)	0 (0.00)	–
			GT + GG	285 (99.65)	138 (100.00)		239 (99.58)	90 (98.90)		46 (100.00)	48 (100.00)	
		Additive model	GT	32 (11.19)	13 (9.42)	0.614	26 (10.83)	8 (8.16)	0.459	6 (13.04)	5 (10.42)	0.692
			GG + TT	254 (88.81)	125 (90.58)		214 (89.17)	90 (91.84)		40 (86.96)	43 (89.58)	
	Allele		G	538 (94.06)	263 (95.29)	0.462	452 (94.17)	172 (95.56)	0.484	86 (93.48)	91 (94.79)	0.701
			T	34 (5.94)	13 (4.71)		28 (5.83)	8 (4.44)		6 (6.52)	5 (5.21)	
rs11572223 (SNP2)	Genotype	Dominant model	C/C	224 (79.43)	242 (75.86)	0.215	185 (79.06)	127 (80.89)	0.442	39 (81.25)	114 (70.81)	0.358
			T/T	11 (3.90)	8 (2.51)		10 (4.27)	3 (1.91)		1 (2.08)	5 (3.11)	
			C/T	47 (16.67)	69 (21.63)		39 (16.67)	27 (17.20)		8 (16.67)	42 (26.09)	
			CC	224 (79.43)	242 (75.86)	0.401	185 (79.06)	127 (80.89)	0.658	39 (81.25)	114 (70.81)	0.152
			CT + TT	58 (20.57)	77 (24.14)		49 (20.94)	30 (19.11)		9 (18.75)	47 (29.19)	
		Recessive model	TT	11 (3.90)	8 (2.51)	0.33	10 (4.27)	3 (1.91)	0.201	1 (2.08)	5 (3.11)	0.71
			CT + CC	271 (96.10)	311 (97.49)		224 (95.73)	154 (98.09)		47 (97.92)	156 (96.89)	
		Additive model	CT	47 (16.67)	69 (21.63)	0.124	39 (16.67)	27 (17.20)	0.891	8 (16.67)	42 (26.09)	0.179
			CC + TT	235 (83.33)	250 (78.37)		195 (83.33)	130 (82.80)		40 (83.33)	119 (73.91)	
	Allele		C	495 (87.77)	553 (86.68)	0.573	409 (87.39)	281 (89.49)	0.372	86 (89.58)	270 (83.85)	0.165
			T	69 (12.23)	85 (13.32)		59 (12.61)	33 (10.51)		10 (10.42)	52 (16.15)	
rs2280275 (SNP3)	Genotype	Dominant model	C/C	225 (66.96)	336 (75.00)	0.048*	182 (66.91)	140 (75.27)	0.154	43 (67.19)	196 (74.81)	0.34
			T/T	16 (4.76)	16 (3.57)		11 (4.04)	5 (2.69)		5 (7.81)	11 (4.20)	
			C/T	95 (28.27)	96 (21.43)		79 (29.04)	41 (22.04)		16 (25.00)	55 (20.99)	
			CC	225 (66.96)	336 (75.00)	0.014*	182 (66.91)	140 (75.27)	0.034*	43 (67.19)	196 (74.81)	0.217
			CT + TT	111 (33.04)	112 (25.00)		90 (33.09)	46 (24.73)		21 (32.81)	66 (25.19)	
		Recessive model	TT	16 (4.76)	16 (3.57)	0.404	11 (4.04)	5 (2.69)	0.438	5 (7.81)	11 (4.20)	0.38
			CT + CC	320 (95.24)	432 (96.43)		261 (95.96)	181 (97.31)		5992.19%	25195.80)	
		Additive model	CT	95 (28.27)	96 (21.43)	0.027*	79 (29.04)	41 (22.04)	0.094	16 (25.00)	55 (20.99)	0.486
			CC + TT	241 (71.73)	352 (78.57)		193 (70.96)	145 (77.96)		48 (75.00)	207 (79.01)	
	Allele		C	545 (81.10)	768 (85.71)	0.014*	443 (81.43)	331 (86.65)	0.035*	102 (79.69)	447 (85.31)	0.118
			T	127 (18.90)	128 (14.29)		101 (18.570	51 (13.35)		26 (20.31)	77 (14.69)	

CAD, coronary artery disease.
The P value of genotype was calculated by Fisher's exact test. *P < 0.05.

Table 3
Multiple logistic regression analysis for CAD patients and control subjects of Uygur population.

	Total			Men			Women		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Dominant model (CC vs CT + TT)	0.279	0.176–0.440	0.001	0.24	0.128–0.457	0.001	0.615	0.291–1.302	0.204
Glu	1.249	1.139–1.370	0.001	1.239	1.092–1.406	0.001	1.374	1.143–1.653	0.001
LDL	0.896	0.799–1.003	0.057	0.912	0.878–1018	0.100	0.416	0.193–0.896	0.025
EH	6.541	3.946–10.842	0.001	9.92	4.83–20.403	0.001	4.002	1.765–9.074	0.001
DM	1.414	0.839–2.384	0.194	1.891	0.895–3.944	0.095	0.86	0.347–2.133	0.746
Smoking	8.18	5.254–12.735	0.001	10.121	5.609–18.264	0.001	0.221	0.028–1.734	0.151

EH, essential hypertension; DM, diabetes mellitus; CAD, coronary artery disease.

disease (CVD) such as hypertension, CAD, heart failure, stroke, cardiomyopathy and arrhythmias has been established [8]. In humans, CYP2J2 acts mainly in converting arachidonic acid to EETs, and EETs were rapidly hydrolyzed by soluble epoxide hydrolase (sEH) to the corresponding dihydroxyeicosatrienoic acid (DHET). DHETs were initially thought to be inactivation products of EETs, but several recent studies indicate that, like EETs, they produce vasodilation and activate smooth muscle BK_{Ca} channels. EETs have been established to have five physiological functions. First, EETs produce vasodilation in a number of vascular beds by activating the smooth muscle large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) [23,31] (Fig. 2). Second, EETs may play a role in the coronary circulation as endothelium-derived hyperpolarizing factors (EDHF) [32]. EDHF possess potent vasodilating effects and hyperpolarize vascular smooth muscle cells (VSMCs) by activating K_{Ca} [23,33,34]. Third, EETs inhibit inflammation responses by decreasing the cytokine-induced endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) and decrease leukocyte adhesion to the vascular wall by inhibiting nuclear factor κB (NF-κB) and IκB kinase [35]. Fourth, EETs have antithrombotic effects by inhibiting platelet adhesion to endothelial cells, inhibiting platelet aggregation, and enhancing the expression and activity of tissue plasminogen activator [36]. Fifth, in the kidney, EETs are important regulators of glomerular filtration by activating Na^+/H^+ exchanger and mediate pressure natriuresis and long-term control of blood pressure [37,38]. Polymorphisms of the CYP2J2 gene may affect the metabolism of arachidonic acid, resulting in an altered synthesis of EETs. In this study, we hypothesized that variability in the gene might affect the risk of CAD. We genotyped three SNPs of the gene in a Han population and an Uygur

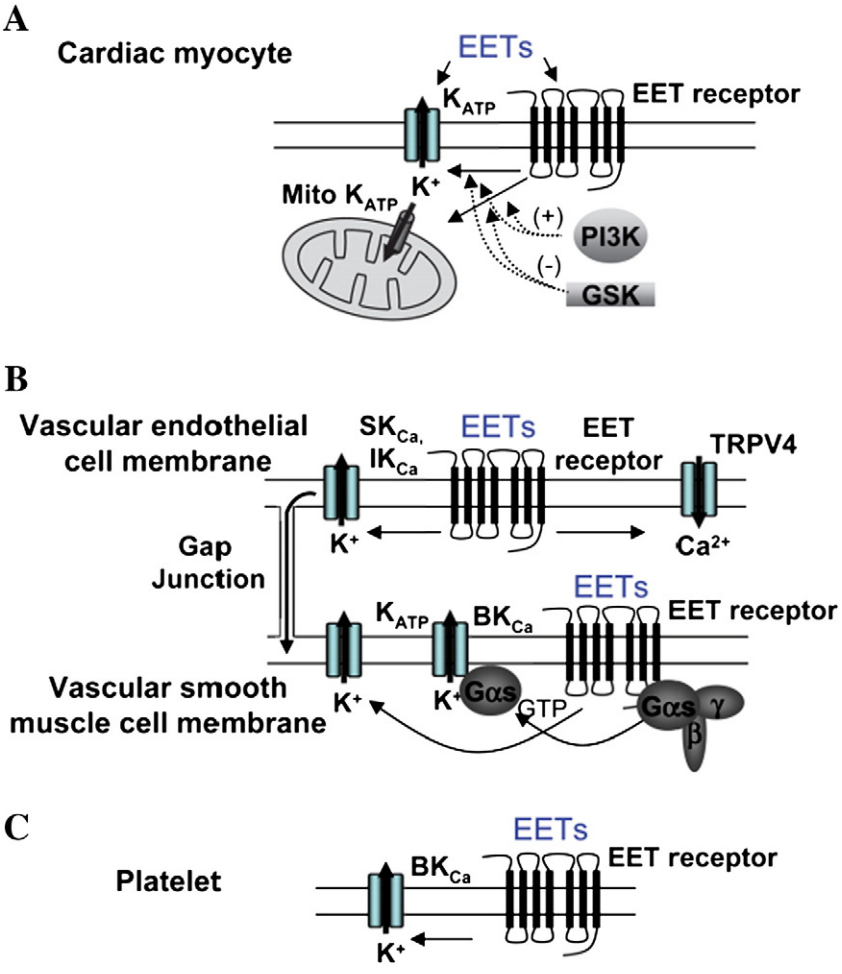

Fig. 2. Schematic of EET interactions with cardiovascularion channels. A: In the cardiac myocyte, EETs activate sarcolemmal or mitochondrial KATP channels. B: In the vasculature, EETs activate endothelial small (SK_{Ca}) or intermediate (IK_{Ca}) conductance calcium-activated channels to cause hyperpolarization, which can be transmitted to the vascular smooth muscle via myoendothelial gap junctions. EETs also activate TRPV4 channels to activate Ca^{2+} influx. In the vascular smooth muscle, EETs activate large conductance, calcium-activated (BK_{Ca}) channels through a G-protein-coupled event. C: In platelets, EETs activate BK_{Ca} channels.

population, and assessed the association between the polymorphism of CYP2J2 gene and CAD using case–control analyses.

In the Uygur population, for total group, there was a significant difference in the genotypic distribution of SNP3 (rs2280275) between CAD patients and control subjects. When analyzing men and women separately, T allele frequency of rs2280275 was higher in CAD patients than in control subjects in men. There was no difference of T allele between CAD patients and control subjects in women. It meant that the risk of CAD was increased with the T allele of rs2280275 in men. For total and men, the dominant model (CC vs CT + TT) was significantly lower in CAD patients than in control subjects, and the significant difference was retained after adjustment for covariates such as Glu, LDL-C, EH, DM, and smoking (Table 3). This indicated that the risk of CAD was decreased with CC genotype of rs2280275 in men of Uygur population.

We found that there was the association between rs2280275 of CYP2J2 gene and CAD only in male subgroup. This may be attributed to sex hormones. Sex hormones such as estrogens protect against oxidative stress and are known to be vasoprotective [39–41]. In addition, there were some researches that reported estrogens protect the EETs against being hydrolyzed by soluble epoxide hydrolase (sEH). [42,43].

Though SNP1 (rs890293) was observed in the proximal promoter region of the CYP2J2 gene, and the polymorphisms caused a loss of transcription factor binding site Sp1, resulting in the synthesis of EETs was reduced. The studies about the association between CYP2J2 polymorphisms (rs890293) and the cardiovascular risk have provided inconsistent results. The study by Spiecker that showed a functionally relevant polymorphism of the CYP2J2 gene (rs890293) independently was associated with an increased risk of coronary artery disease [14]. This result was supported by the study of Ping Yin Liu showing the polymorphism of CYP2J2 (rs890293) was an important risk factor for the development of MI in younger groups in Tainans [25]. In addition, a low risk of CAD was reported by Lee in African-Americans carrying CYP2J2 variant alleles (rs890293), but no significant association was observed in Caucasians [26]. There were several studies suggesting no significant association between the polymorphism of CYP2J2 (rs890293) and CAD or MI [27,29], and in a recent large sample case–control study and meta-analysis, they also have not observed any significant association between common polymorphisms within CYP2J2 genes and CAD, whether using methods of single-locus analysis or haplotype-based analysis [28]. Our study was consistent with the studies [27,28], showing no significant association between the polymorphism of CYP2J2 (rs890293) and CAD. There may be differences in populations and geographical factors to explain some differences. For the SNP2 (rs11572223), our result was consistent with Zeng Jie and his colleagues that the genotype and the allele distributions of rs11572223 were not different between the CAD patients and control subjects [44].

5. Conclusion

In conclusion, we found that rs2280275 may be a novel polymorphism of the CYP2J2 gene associated with CAD in Uygur male population in China. The CC genotype of rs2280275 in CYP2J2 gene could be a protective genetic marker of CAD and T allele may be a risk genetic marker of CAD in men of Uygur population in China. Certainly it needs a large number of clinical samples to study further in China.

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